

REMARKS

Reconsideration and allowance are respectfully requested. Claims 40-61 are pending. The amendments are fully supported by the original disclosure and, thus, no new matter is added by their entry. Claims 40, 51 and 55 are amended to explicitly recite implicit features of the claimed invention: *i.e.*, “presenting to the T cells a T cell-activating peptide derived from the pathogen in the absence of any antigen presenting cells pre-cultured with said peptide.” Claims 49-50 are amended to address the Examiner’s rejections for lack of active steps. Support for these amendments can be found in the specification, for example, at page 3, lines 23-28, and the examples. Claims 54 and 58 are amended to better reflect the antecedents in the independent claims from which they depend. New claims 59-61 are supported by the specification at page 4, lines 3-4. These amendments do not constitute new matter or narrow claim scope. Entry of the amendments will reduce the issues on appeal.

To establish a case of *prima facie* obviousness, all of the claim limitations must be taught or suggested by the prior art. See M.P.E.P. § 2143.03. A claimed invention is unpatentable if the differences between it and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art. *In re Kahn*, 78 USPQ2d 1329, 1334 (Fed. Cir. 2006) citing the legal standard provided in *Graham v. John Deere*, 148 USPQ 459 (1966). The *Graham* analysis needs to be made explicitly. *KSR v. Teleflex*, 82 USPQ2d 1385, 1396 (2007). It requires findings of fact and a rational basis for combining the prior art disclosures to produce the claimed invention. See *id.* (“Often, it will be necessary for a court to look to interrelated teachings of multiple patents . . . and the background knowledge possessed by a person having ordinary skill in the art, all in order to determine whether there was an apparent reason to combine the known elements in the fashion claimed by the patent at issue”). The use of hindsight reasoning is impermissible. See *id.* at 1397 (“A factfinder should be aware, of course, of the distortion caused by hindsight bias and must be cautious of arguments reliant upon *ex post* reasoning”). Thus, a rejection under Section 103(a) requires “some rationale, articulation, or reasoned basis to explain why the conclusion of [*prima facie*] obviousness is correct.”

Kahn, 78 USPQ2d at 1335; see *KSR*, 82 USPQ2d at 1396. An inquiry should be made as to “whether the improvement is more than the predictable use of prior art elements according to their established functions.” *Id.* at 1396. But a claim which is directed to a combination of prior art elements “is not proved obvious merely by demonstrating that each of its elements was, independently, known in the prior art.” *Id.* at 1396. Finally, a determination of *prima facie* obviousness requires a reasonable expectation of success. See *In re Rinehart*, 189 USPQ 143, 148 (C.C.P.A. 1976).

The Examiner has failed to establish a *prima facie* case of obviousness. To establish a *prima facie* case of obviousness, (1) there must be some suggestion or motivation, either in the documents themselves or in the knowledge generally available to one of ordinary skill in the art, to modify or combine their disclosures; (2) there must be a reasonable expectation of success; and (3) the prior art document (or documents when combined) must teach or suggest all of the claim limitations. M.P.E.P. § 2143.03. The cited documents, considered separately or in combination, do not provide one of ordinary skill in the art an acceptable reason to modify or combine them as proposed in the Office Action, or a reasonable expectation of success in arriving at Applicants' claimed invention, and the combination of these documents does not teach or suggest all limitations of the rejected claims.

The obviousness rejections rely on a misapplication of Hagiwara's disclosure to use fresh T cells (*i.e.*, cells not cultured *in vitro*) to enumerate active T cells by detecting IFN- γ produced by the T cells in an ELISPOT assay. Office Action at 3-10. To summarize, Hagiwara says not one word about antigen- or pathogen-specific responses, or about diagnosing or monitoring infection with an intracellular pathogen. This document provides no basis for testing fresh lymphocytes for the presence of active effector cells specific for an intracellular pathogen.

Claims 40 And 43-50 Are Not Obvious Over Miyahira In View Of Hagiwara

Claims 40 and 43-50 were rejected under Section 103(a) as allegedly unpatentable over Miyahira *et al.* (*J. Immunol. Meth.* 181:45-54, 1995; hereinafter Miyahira) in

view of Hagiwara *et al.* (*AIDS Res. Hum. Retrovir.* 12:127-133, 1996; hereinafter “Hagiwara”). Applicants traverse for the following reasons.

Independent claim 40 recites, “A method of diagnosis or monitoring of infection with an intracellular pathogen in an individual wherein peptide-specific effector T cells are enumerated.” The peptide is derived from an intracellular pathogen. The T cells are fresh: *i.e.*, they have not been cultured. The T cells have been pre-sensitized to the peptide *in vivo*. And the T cells are presented with the peptide *in vitro* in the absence of antigen presenting cells (APCs), which have been pre-cultured with said peptide.

By contrast, Miyahira discloses a conventional ELISPOT assay to determine the number of antigen-specific CD8⁺ T cells based on the detection of IFN- γ secretion by single cells after their stimulation with antigen. Miyahira at 45. In accordance with its ELISPOT assay, (i) antigen-specific CD8⁺ T cells (*e.g.*, cloned murine YA26 cells or spleen cells removed from immunized mice) are placed in wells coated with an antibody to IFN- γ , (ii) pre-cultured APCs (*e.g.*, P815 cells) pulsed with a peptide having an amino acid sequence of an epitope of the antigen are added to the wells, (iii) the T cells are incubated with the APCs for 24-28 hours, and (iv) IFN- γ secretion by the T cells are detected. Miyahira at 46-48.

Thus, two important differences distinguish Miyahira from the claimed invention. First, Miyahira relies on pre-cultured cells. In contrast, in accordance with the claimed invention, there are no pre-cultured APCs present. Second, Miyahira incubates lymphocytes with APCs for at least 24 hours in the ELISPOT assay, which results in activation of resting or memory cells. In contrast, the present invention provides for an incubation time that only elicits IFN- γ production from active effector cells. Miyahira would not identify the effector T cells in a fluid sample with any reasonable expectation of success.

Hagiwara reports, “The number of peripheral blood mononuclear cells (PMBC) spontaneously secreting both type 1 and type 2 cytokines was significantly reduced in HIV-infected patients versus controls” based on using ELISPOT assays. Hagiwara at 127. The focus of the analysis was “cytokine-producing cells that were actively participating in ongoing immune responses in HIV-infected individuals.” Hagiwara at 127-128. In some cases, phytohemagglutinin (PHA) was added during the ELISPOT assay to

analyze the effect of *in vitro* mitogen stimulation on cytokine production by PBMC, and it was found that “the frequency of PBMC capable of rapidly secreting IL-2, IL-4, or IFN- γ following PHA treatment was 2 to 3-fold lower in patients infected with HIV than normal volunteers.” Hagiwara at 128.

There Is No Basis to Combine Miyahira with Hagiwara

According to the Examiner, “Miyahira discloses the quantification of antigen-specific CD8⁺ T cells specific for the epitope SYVPSAEQI of a rodent malaria antigen using an ELISPOT assay” but “does not disclose the use of fresh T cells that have not been cultured *in vitro*.” Office Action at 4. “Hagiwara teaches that ELISPOT results are divergent when studying PBMCs that have been cultured and stimulated *in vitro*.” *Id.* As set forth below, there is no reason to combine Miyahira with Hagiwara.

Applicants note that Miyahira discloses determination of antigen-specific splenic CD8⁺ T cells by using freshly isolated spleen cells from mice immunized with a recombinant vaccinia virus stimulated with antigen-pulsed APCs in a conventional ELISPOT assay. Miyahira at 47 and 50-52. The spleen cells are contacted with pre-cultured antigen-pulsed P815 APCs in the assay. P815 cells were incubated with the synthetic peptide SYVPSAEQI for one hour at 37°C, followed by repeated washings with culture medium. Miyahira at 46. Miyahira emphasizes, “One of the steps that appears to be crucial for the detection of CD8⁺ T cell derived IFN- γ spots is the need for mixing the CD8⁺ T cells and peptide-coated target cells just prior to placing them in the ELISPOT wells, avoiding the cell transfer and consequent loss of secreted IFN- γ ” (emphasis added). Miyahira at 53. Miyahira states that use of pre-cultured APCs ensures that the APCs express class I and not class II MHC molecules. But since there are APCs that express both class I and II MHC molecules present in the fluid sample, the cultured cells must be pulsed with the antigen and washed prior to adding them to the cells in the fluid sample. Miyahira at 53. Exogenous APCs, which are pulsed in culture with peptide and then washed to remove extra peptide, are required in Miyahira to detect a pathogen-specific immune response. Therefore, Miyahira teaches away from the requirement of Applicants’ claimed invention to present a peptide derived from a pathogen to peptide-specific T cells in the absence of pre-cultured APCs.

Moreover, the temporal differences between Miyahira and Applicants' claimed invention are non-obvious and significant. In Miyahira, responder cells are pre-mixed with antigen-pulsed APCs (requiring a finite time period) and subsequently incubated for 24-28 hours in ELISPOT wells. Miyahira at 46 and 53. Thus, the minimum incubation time required in Miyahira exceeds the maximum incubation time of the pending claims. The latter limitation of Applicants' claimed invention ensures that the assay detects active effector cells and not quiescent cells. See the specification at page 3, line 29, to page 4, line 4. Furthermore, examination of Figure 7 in Miyahira demonstrates that incubation of the cell sample with antigen-pulsed APC in long-term culture greatly amplifies the response (compare the "fresh spleen cells" graph to the "cultured spleen cells" graph). Thus, based on Miyahira, one of ordinary skill in the art would be even more inclined to culture the cells in the sample with antigen-pulsed APCs for a number of days to amplify the response instead of using fresh cells as required by the pending claims.

Hagiwara teaches away from modifying Miyahira to arrive at Applicants' claimed invention. Hagiwara teaches that "the frequency of PBMC capable of rapidly secreting . . . IFN- γ following PHA treatment was 2- to 3-fold lower in patients infected with HIV than normal volunteers." Hagiwara at 128. Unlike the situation in which a specific peptide is presented to the T cells to stimulate IFN- γ release by antigen-specific (and thus pathogen-specific) effector T cells according to the claimed invention, Hagiwara uses cytokine secretion as an indicator of general immune health: "This approach is uniquely suited to determining whether a physiologically relevant shift from type 1 to type 2 cytokine production has occurred, since it identifies all cytokine-secreting cells in the immune milieu rather than focusing on cell of a single phenotype or those responsive to stimulation *in vitro*." Hagiwara at 131. If cells are stimulated, they are stimulated with the mitogen phytohemagglutinin (PHA), and results in stimulation of cytokine production by all PBMC. Hagiwara's report of decreased cytokine production in infected patients relates to their general immune health, not to specific immunity to HIV, and thus has nothing to do with the claimed invention.

Therefore, there is no reasonable basis to combine Hagiwara with Miyahira, or use such a combination to reject the claims of the present invention for obviousness.

There Is No Reasonable Expectation of Success in Making the Claimed Invention

In view of the disclosures of Miyahira and Hagiwara that teach away from Applicants' claimed invention, there is no reasonable expectation of success in combining the two cited documents in arriving at the pending claims.

Miyahira And Hagiwara Do Not Teach Or Suggest All Limitations of the Claims

Miyahira and Hagiwara, considered in combination, do not teach or suggest all the limitations in claim 40 or claims depending therefrom. The documents in combination do not teach "[a] method of diagnosis or monitoring of infection with an intracellular pathogen in an individual wherein peptide-specific effector T cells are enumerated." See claim 40. It is also important to note in this regard that Hagiwara's test does not diagnose infection with HIV. If it is diagnostic at all, it only diagnoses immunodeficiency. Further, for reasons Hagiwara could not explain, the test does not provide any effective monitoring of HIV infection: "The magnitude of this decrease did not correlate with disease severity." Hagiwara, Abstract. Miyahira also fails to teach a method of diagnosis or monitoring infection with an intracellular pathogen in an individual, which comprises presenting a peptide derived from the pathogen to T cells from the individual in the absence of pre-cultured APCs. As conceded by the Examiner, Hagiwara fails to teach a method of diagnosis or monitoring infection with an intracellular pathogen in an individual, which comprises presenting a peptide derived from the pathogen to the T cells. Office Action at 4-5. Thus, combining these two documents does not teach or suggest a method of diagnosis or monitoring infection with an intracellular pathogen in an individual, which comprises presenting a peptide to the T cells in the absence of pre-cultured APCs as required by the pending claims.

In summary, independent claim 40 and claims depending therefrom are not obvious over Miyahira in view of Hagiwara. Therefore, Applicants respectfully request withdrawal of the Examiner's rejection of claims 40 and 43-50 under 35 U.S.C. 103 as being unpatentable over Miyahira in view of Hagiwara.

Claims 40-43, 45-48 and 51-58 Are Not Obvious Over Surcel In View of Sørensen and Hagiwara

Claims 40-43, 45-48 and 51-58 were rejected under Section 103(a) as allegedly unpatentable over Surcel *et al.* (*Immunol.* 81:171-176, 1994; hereinafter “Surcel”) in view of Sørensen *et al.* (*Infect. Immun.* 63:1710-1717, 1995; hereinafter “Sørensen”) and Hagiwara. Applicants traverse for the following reasons.

Independent claim 40 recites, “A method of diagnosis or monitoring of infection with an intracellular pathogen in an individual wherein peptide-specific effector T cells are enumerated.” Independent claim 51 is directed to a method similar to that in claim 40 except the pathogen is *M. tuberculosis*. Independent claim 55 relates to a method similar to that in claim 51 except the incubation time is from 4 to 24 hours. Among the relevant limitations of the claimed invention, the peptide is derived from an intracellular pathogen; the T cells are fresh, or have not been cultured; the T cells have been pre-sensitized to the peptide *in vivo*; and the T cells are presented with the peptide *in vitro* in the absence of pre-cultured antigen presenting cells (APCs).

In Surcel, “Proliferation and cytokine production profiles by blood mononuclear cells in response to *in vitro* stimulation with mycobacterial antigens were compared in patients with active tuberculosis and in sensitized healthy controls” based on detection of IL-4 and IFN- γ at single-cell level using the ELISPOT assay. Surcel at 171. PMC were incubated for 72 hours in the presence of a mycobacterial antigen before being transferred to anti-IFN- γ antibody-coated plates. Surcel at 172. Thus, Surcel lacks the “not cultured *in vitro*” and the temporal limitations of Applicants’ claimed invention.

Sørensen discloses ESAT-6 is a low molecular mass T cell antigen secreted by *M. tuberculosis*, and “[n]ative and recombinant ESAT-6 are immunologically active in that both elicited a high release of gamma interferon from T cells isolated from memory-immune mice challenged with *M. tuberculosis*.” Sørensen at 1710. But Sørensen also does not teach or suggest using ESAT-6 in a diagnostic assay in accordance with Applicants’ claimed invention: fresh cells and the temporal aspects of the pending claims.

The teachings of Hagiwara are discussed above.

There Is No Basis To Combine Surcel with Sørensen and Hagiwara

According to the Examiner, “Surcel uses the ELISpot assay to measure effector T cells that produce IFN- γ ” and “Sørensen discloses that native and recombinant ESAT-6 elicited a high release of IFN- γ from T cells isolated from memory-immune mice challenged with *M. tuberculosis*.” Office Action at 7. As explained below, however, there is no suggestion or motivation to combine Surcel with Sørensen and Hagiwara.

But Surcel teaches away from Applicants’ claimed invention. Surcel discloses that freshly isolated PBMC should be incubated in 96-well plates for 72 hours prior to incubation for the ELISPOT assay. Surcel at 172. This reinforces the teachings of Miyahira, and further confirms that the prior art generally believed that IFN- γ ELISPOT assays require culturing T cells in the presence of antigen for long periods, in contrast to the pending claims. As conceded by the Examiner, “The incubation of T cells with T cell activating peptide for 72 hours would allow memory T cells to proliferate, thus the measurement of IFN-gamma producing T cells would include both the memory T cells and effector T cells.” Office Action at 8. In contrast, as taught in Applicants’ specification, an assay in accordance with the pending claims:

measures peptide-specific effector cells directly, without requiring these cells to proliferate *in vitro*. The short duration of the assay also eliminates the possibility that the cells may be becoming activated *in vitro*; it therefore measure effector function that is present *in vivo*.

Specification, page 5, lines 20-24. It is improper to simply disregard these advantages of the pending claims, which cannot be achieved following Surcel, in analyzing obviousness (or, in the present case, lack of obviousness) of Applicants’ claimed invention which is a direct result of using fresh cells and a short incubation time.

Sørensen merely teaches that ESAT-6 is an immunogen from *M. tuberculosis* with potential value as a vaccine candidate. Sørensen at 1710. To the extent ESAT-6 demonstrated activity in memory immunity, it was in a mouse model of tuberculosis. *Id.* at 1716. Sørensen says nothing about using ESAT-6 in an ELISPOT assay to diagnose or monitor an infection by an intracellular pathogen, much less about doing so in a way that ensures detection of effector cells. Most importantly, although ESAT-6 protein is a

source for ESAT-6 peptides, the document does not provide a specific sequence of an immunodominant peptide.

Hagiwara teaches away from modifying Surcel, alone or in combination with Sørensen, to arrive at the claimed invention: *i.e.*, a method of diagnosis or monitoring infection with an intracellular pathogen in an individual comprising presenting a T cell-activating peptide derived from a pathogen to T cells from the individual. Hagiwara teaches that “the frequency of PBMC capable of rapidly secreting . . . IFN- γ following PHA treatment was 2- to 3-fold lower in patients infected with HIV than normal volunteers.” Hagiwara at 128. Unlike the situation in which a specific peptide is presented to the T cells to stimulate IFN- γ release by antigen-specific (and thus pathogen-specific) effector T cells according to the claimed invention, Hagiwara uses cytokine secretion as an indicator of general immune health. “This approach is uniquely suited to determining whether a physiologically relevant shift from type 1 to type 2 cytokine production has occurred, since it identifies all cytokine-secreting cells in the immune milieu rather than focusing on cell of a single phenotype or those responsive to stimulation *in vitro*.” Hagiwara at 131. If cells were stimulated, they were stimulated with the mitogen PHA, resulting in stimulation of cytokine production by all PBMC. Hagiwara at no point describes stimulation with a pathogen-specific peptide antigen (like an ESAT-6 peptide). Hagiwara’s report of decreased cytokine production in infected patients relates to their general immune health, not to specific immunity to HIV, and thus has nothing to do with the claimed invention.

Thus, based on Surcel, the incubation time for the assay would be too long and, based on Hagiwara, no antigen-specific response would be measured. There is no objective basis to predict which document to modify, much less how to modify either document in light of the other to achieve some desired outcome. There is no reasonable basis to combine Hagiwara with either Surcel or Sørensen, or to use the combination to render the pending claims obvious.

There Is No Reasonable Expectation Of Success

In view of the teachings in Surcel and Hagiwara away from the claimed invention, there is no reasonable expectation of success in combining Surcel with Sørensen and

Hagiwara in arriving at Applicants' claimed invention. In particular, there is no basis to conclude that an antigen-specific release of IFN- γ would be possible without either pre-cultured APC cells or long-term *in vitro* culture of the cells in the fluid sample, or both. Indeed, there is no reasonable expectation that such an assay could be used to diagnose or monitor infection with *M. tuberculosis* (or any pathogen) since the combined documents do not establish any such result.

Surcel, Sørensen and Hagiwara Do Not Teach Or Suggest All The Limitations

Surcel, Sørensen and Hagiwara, considered in combination, do not teach or suggest all the limitations in the claims. As noted above, the combination of documents fails to teach diagnosis or monitoring of *M. tuberculosis* infection. It is important to note in this regard that Hagiwara's test does not diagnose infection of an individual by HIV; it only diagnoses an immunodeficiency condition. Furthermore, for reasons Hagiwara could not explain, the test does not provide any effective monitoring of HIV infection: "The magnitude of this decrease did not correlate with disease severity." Hagiwara, Abstract. Furthermore, the combined documents fail to teach incubation for a time to permit interferon- γ release by only those T cells that have been pre-sensitized *in vivo* to the T cell-activating peptide and are capable of immediate effector function without the need to effect division/differentiation by *in vitro* culture in the presence of the T cell-activating peptide.

In summary, independent claims 40, 51 and 55 and claims depending therefrom are not obvious over Surcel in view of Sørensen and Hagiwara. Therefore, Applicants respectfully request withdrawal of the Examiner's rejection of claims 40-43, 45-48 and 51-58 under 35 U.S.C. 103 as being unpatentable over Surcel in view of Sørensen and Hagiwara.

Applicants submit that the claims are in condition for allowance and earnestly solicit an early Notice to that effect. The Examiner is invited to contact the undersigned if any further information is required.

Respectfully submitted,

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